Dynamic feature of flavonoids content in different organs of larch (*Larix gmelinii*)

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Abstract: Flavonoids in plants is very important in its ecological role and economic value. The dynamic features of flavonoids content in different organs of larch (*Larix gmelinii*) at different light and temperature conditions were investigated in this study. Results showed that the order of flavonoids content in different organs from high to low was 7.78% (stem bark) > 2.79% (leaves) > 1.72% (branches) > 1.19% (stem xylem)and different organs had a great seasonal variation in flavonoids content, but the change of flavonoids content at different temperature was not obvious in different organs., The content of flavonoids in barck had, a positive correlation with temperature (R^2=0.75), but that in other organs had slight variation with the change of temperatures. For all the tested organs, the flavonoids content in summer and autumn was approximately 3-4 times higher than in spring and winter. This is attributed to the great stress from environmental physical variables such as UV radiation, high temperature that induce the accumulation of flavonoids. The flavonoid content of sun leaves was evidently higher than that of shade leaves, and leaves at upper part of canopy had a higher flavonoids content compared with that at other parts. This result indicates that sun radiation could improve flavonoids production in leaves (R^2=0.76). The flavonoids may actively evolve in plant defenses to environmental stress, protecting larch from the damage of high temperature and radiation, and its main function is different in different organs.

Keywords: *Larix gmelinii*; Flavonoids; Dynamic features; Environmental stress
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Introduction

Flavonoids in plants has an important role in the protection of plants against insects and pathogens (Harborne *et al.* 1988), UV light damage defense (Graham 1991), and thermal stress (Wollenweber 1993). The UV-B defense mechanism of flavonoids in the plant was well reported by some scientists. According to their studies, higher levels of flavonoids occurred in leaves that were exposed to greater light intensity. Waterman (1988) and Dudt (1994) have well manifested this variation of plants growing in different light conditions. Needles of evergreen conifer have UV resistant surface layers that protect them from damage (Day *et al.* 1993). Deciduous conifers such as western larch (*Larix occidentalis*) may be more sensitive to UV-B under some conditions (Krol *et al.* 1995), and needles of mature trees are highly effective at screening UV-B in the epidermis (Day *et al.* 1992). The ability of plant resistance to UV-B radiation is correlated with the flavonoids in UV resistant surface layers. UV-B radiation has been found to generate free radicals in plant cells (Hideg *et al.* 1996), and flavonoids can clean out harmful radicals. So an increase in the concentrations of efficient flavonoids antioxidant would be beneficial to plant. Acting as radical-scavenging antioxidants, flavonoids may lead to specific accumulation during UV-B stress (Cooper *et al.* 1998).

Some reports demonstrated that thermal stress induced the production of phenolic compounds by activating their biosynthesis as well as inhibiting their oxidation, such as flavonoids and phenylpropanoids (Dixon *et al.* 1995; Bharti *et al.* 1997). This could be considered as an acclimation to thermal stress. For example thermal stress in tomato and watermelon caused accumulation of flavonoids and other soluble phenolics (Rivero *et al.* 2000). However, more studies are needed to confirm this thermal acclimation.

Larch (*Larix gmelinii*) is widely distributed in northern hemisphere; especially that it is an important afforestation species in Northeast China (Wang *et al.* 2005). Studies on flavonoids of larch have mainly been used for seedlings exposed under controlled conditions in greenhouse or growth chambers (Linda 2004; Cen *et al.* 1993). However, the ecological function of flavonoids in larch in multi-season field is less known. Therefore the aims of this study are, firstly, to determine the dynamic change of flavonoids, then to discuss the function difference of flavonoids in different organs (leaf and bark) and the flavonoids response to light environment and thermal stress.

Materials and methods

Study sites

A larch stand planted in 1969 at Laoshan station (45°20′N, 127°34′E) in Northeast China was selected as our study sites. The altitude is approximately 340 m above sea level. The mean annual precipitation is 723 mm and the non-frost period is 120–140 d. The annual mean air temperature is 2.8 °C. This region is characterized by a typical continental climate and soil is characterized as dark brown forest soil.

Plant materials

L. gmelinii trees with approximately 20-m height and 17.2-cm (4.5SD) diameter at breast height (DBH) were selected. Stem samples (bark and stem xylem) were collected by chisel and hammer on five healthy trees per month from Mar. 2003 to Mar. 2004, respectively. Sampling depth is about 4 cm in bark and xylem. Branches (Mar. 2003–Feb. 2004) about 5 mm in diameter

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at the height of 20 m above the ground were sampled for analysis of seasonal dynamics. Leaves of four healthy trees were sampled at height of 20, 18, 17, 16, 14, and 13m in June 20, 2003 for analyses of light influences on flavonoids. Samples were dried at room temperature, grounded and stored at −18℃ until chemical extraction.

Extraction and chemical analyses of flavonoids

Before analysis, ground samples of 100 mg were weighted exactly and then extracted with 10-mL ethanol of 95% in a screw cap tube in 45 ℃ ultrasonic water bath for 4 h. And then the mixture was centrifuged for 10 min and the supernatant was used in the analysis. NaNO3 (1.0 mL 5%) reagent was added into the tube with 0.5 mL of the supernatant, and was shaken until mixed well. After 6 min, the 1.0-mL Al(NO3)3 of 10% was added to the mixture and then placed there for 6 min. Then 10 mL 5% NaOH was added and fixed to 25 mL with distilled water in volumetric flask. After 15 min, the mixture absorbance at 500 nm was read by spectrophotometer (Unico 2100, China). The standard was rutin (Sigma Corp, USA).

Temperature at the stem and light intensity measurement

The long-term recording of temperature at the stem around the year at the depth of 1 cm was automatically measured by a mini-thermometer system (RTW-30s, Espec Mic Corp. Aichi, Japan). The frequency of data recording was twice per hour.

A PAR (photosynthetic active radiation) sensor of Li6400 was used to determine the light intensity at heights of 20, 18, 17, 16, 14, and 13 m above ground. For each height, 20 data was measured. SPSS 11.0 (SPSS.USA) was used in the statistical analysis of data.

Results and analysis

Comparisons of flavonoids content in different organs

Flavonoids content had significant difference among different organs. The highest content of flavonoids was observed in bark (7.78%), which was 5 times that in stem xylem, indicating that flavonoids was mainly accumulated in the bark. The order of flavonoids content in different organs was as follows: bark (7.78%) >leaves (2.79%) >branches (1.72%) >stem xylem (1.19%), ($P < 0.05$), (Fig.1).

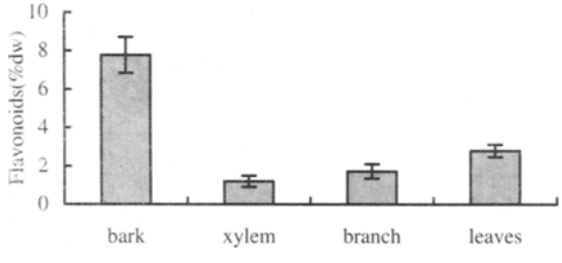

Fig. 1 Content of flavonoids in different organs of larch ($P < 0.05$, vertical bars show the standard error of mean value)

Seasonal change of flavonoids content in different organs

With the season changing (Figs. 2, 3, 4, 5), flavonoids content in different organs (bark, stem xylem, leaves, 1-yr-old branch, 2 yr-branch, 3-yr-branch) of larch showed an obvious seasonal variation. The peak of flavonoids content in different organs was observed during summer and autumn, while in spring and winter the flavonoids content was low.

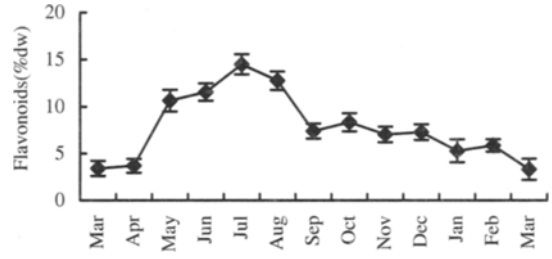

Fig. 2 Seasonal variation of flavonoids content in bark of larch (Vertical bars show the standard error of mean value)

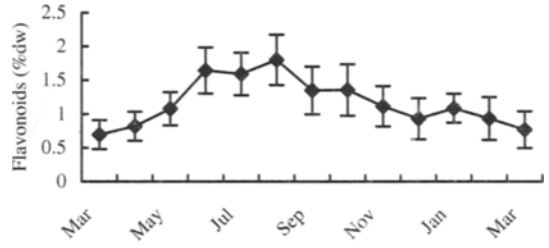

Fig. 3 Seasonal variation of flavonoids content in stem xylem of larch

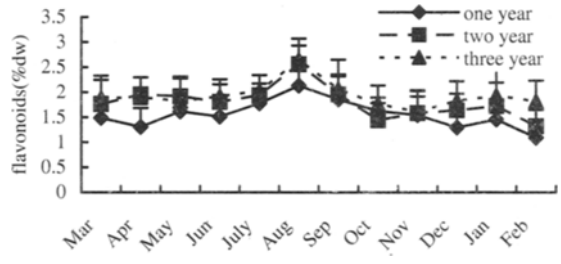

Fig. 4 Seasonal variation of flavonoids content in the branches with different ages of larch

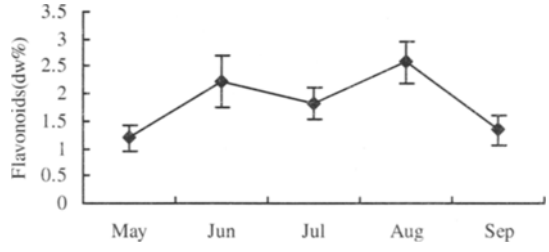

Fig. 5 Seasonal change of flavonoids content in leaves of larch

Spatial change of light intensity in leaves

The leaves light intensity in the upper layers of canopy was higher than that in the lower layers, at the same time, the light intensity in sun leaves was higher than that in the leaves of other direction of canopy (Fig. 6 and Fig. 7).

Spatial change of flavonoids content in leaves

The flavonoids content in the upper layer leaves of canopy was higher than that in the lower layers (Fig. 6). With the leaves height decreasing, the leaves flavonoids decreased. The content of the flavonoids in the sun leaves was 1.5 times higher than that in shaded leaves (Fig. 7). Flavonoids content was positively re-

lated with light intensity (R2=0.76), (Fig. 8).

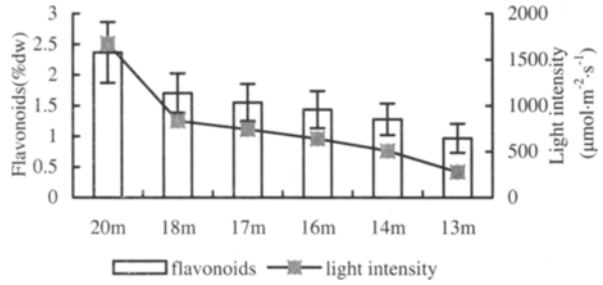

Fig. 6 Relationship between variation of light intensity and flavonoids content in leaves at different height

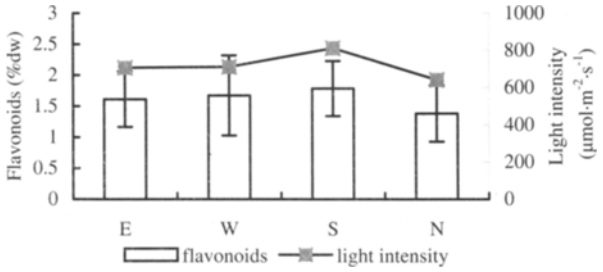

Fig. 7 Relationship between variation of light intensity and leaves flavonoids content at different direction

(E: East; W: West; S:South; N: North; The vertical bars show the standard error of mean value)

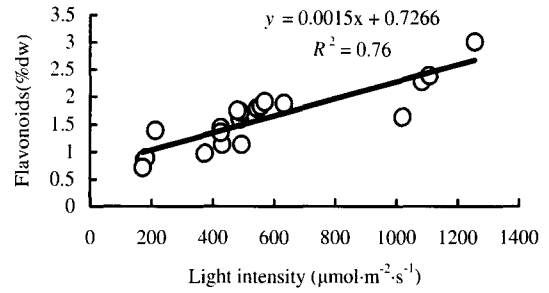

Fig. 8 Correlation between light intensity and flavonoids content in leaves

Discussion

Flavonoids content in different organs of larch with different temperature

In this article, flavonoids content in larch was affected by environmental changes. Content of flavonoids had a peak in summer and autumn, which was three to four times higher than the content measured in spring and winter.

In summer, the plant suffers the greater stress from environment, such as UV irradiation, high temperature, which may lead to the accumulation of flavonoids. Previous studies showed that flavonoids might be accumulated in plants mainly in the following two ways: (1) in response to an environmental stress; (2) in response to cell damage or infection by a pathogen microorganism (McClure *et al.* 1975). Since we chose healthy trees as object, the effect of insects and pathogens can be ignored.

High temperature may be one of the important factors affecting the change of flavonoids content, because thermal stress induces the production of phenolics compounds by activating their biosynthesis as well as inhibiting their oxidation, such as flavonoids and phenylpropanoids (Panagopoulos *et al.* 1992; Cen *et al.* 1993). In our study, flavonoids are mainly accumulated in the bark of larch (Fig. 1). With temperature increasing, a positive correlation between the flavonoids content and temperature were found (R^2=0.75), but slight temperature dependency of flavonoids content was observed in other organs (Table 1). These results indicated that flavonoids in the bark of trunk and other organs may be sensitive to seasonal temperature changes.

Table 1. The correlation between temperature and content flavonoids (%dw) in different organs

Organ	Regression equation	R^2	P-value
1-yr-old branch	y = 0.014x + 1.4819	0.41	0.02
2-yr old branch	y = 0.0158x + 1.7152	0.40	0.03
3-yr old branch	y = 0.0094x + 1.8826	0.22	0.08
bark	y = 0.2495x + 7.0006	0.75	0.02
Stem xylem	y = 0.0179x + 1.1081	0.45	0.01
leaves	y = 0.1117x - 0.1278	0.46	0.02

Solar radiation, in particular UV radiation, not only stimulate but also induce the flavonoids compounds (McClure *et al.* 1975; Wellmann *et al.* 1983; Zaprometov *et al.* 1988). Bell *et al.* (1980) proposed that the synthesis of flavonoids should be regarded as a defense mechanism of the plant against stress. This hypothesis is supported by the observation that UV light is a very potent inducer of flavonoids production in plants (Chapell *et al.* 1984; Vogt *et al.* 1991; Ziska *et al.* 1993). Flavonoids can absorb in the UV-region of the spectrum (McClure *et al.* 1975) and are capable of protecting plant cells from the harmful effects of UV-radiation.

Growth status may be linked to accumulation of flavonoids. In autumn, plant growth is ceased and photosynthate are reallocated to the secondary metabolites, such as flavonoids, tannins and other phenolics (Wang *et al.* 2000), therefore more flavonoids accumulate, then peak in autumn. In spring, photosynthesis would intensify when leaves are hardened. Greater carbohydrates are allocated to primary metabolites in preparation for growth of plant, while accumulation of secondary metabolites are declined. Hence flavonoids content is low over the period of spring (Wang *et al.* 2000). The low flavonoids content in winter may be due to the lower photosynthetic photon flux density as well as low temperature. These discoveries indicated that flavonoids content in different organs of larch had a large seasonal variation (especially solar radiation and temperature).

Light density regulation on leaf flavonoids content

Leaf chemical traits may also differ along a vertical gradient from the upper to the lower sections of a tree crown, because a canopy can respond to light variation by producing specialized sun and shade leaves (Lowman *et al.* 1985, Hollinger *et al.* 1989, Ellsworth *et al.* 1993). From Fig. 6 and Fig. 7, we can see that the leaves light intensity in the upper layers of canopy are higher than that in the lower layers, at the same time, the light intensity in sun leaves are higher than that in the leaves of other direction.

We have found that leaf flavonoids content may also be affected by the location in the canopy. The flavonoids content in the upper layers of canopy with higher light intensity was more

than that in the lower layers with low light intensity (Fig. 6). With the height of leaves decreased, the content of flavonoids in leaves decreased. Flavonoids content in sun leaves was high, whereas in shade leaves was low (Fig.7), (Mole 1988; Dudt 1994). The possible reason is that conifer needles are highly effective in screening ultraviolet-B radiation (280-320 nm) and this ability is mainly attributed to the presence of flavonoids and hydroxycinnamic acids in the epidermal tissue. The authors suggested that production of flavonoids by the epidermal cells of the leaves might serve to prevent the damaging effects of excessive UV light reaching internal tissues as UV filters (Day *et al.* 9921). In our results, flavonoids content was positively related to light intensity (R2=0.76). We have provided data of light intensity of solar radiation in our results. The higher light intensity of solar radiation means the higher UV radiation (Zhou *et al.* 1984). Greater UV radiation induced the accumulation of flavonoids. Therefore leaves in sunny environment could absorb more UV radiation and induced more flavonoids, which could protect leaves from photodamages (Mole 1988; Dudt 1994). These results showed that flavonoids content in the epidermal tissue of leaves was mainly response for building resistance to UV radiation stress.

Conclusions

During the normal processes of growth and development, plants are subjected to different types of stress. The results of these stress induced the accumulation of flavonoids. (1) With season changing, the peak of flavonoids content in different organs occurred in summer and autumn. This may be attributed to the great stress from environmental physical variables such as UV radiation, high temperature which induce the accumulation of flavonoids. (2) Different organs of larch had significant different flavonoids content. The flavonoids content in stem bark (7.78%) was much more than that in other organs, indicating that flavonoids were mainly accumulated in the bark. (3) The flavonoids may actively evolve in plant defenses to environmental stress, protecting larch from the damage of high temperature and radiation, and its main function is different in different organs The flavonoids in leaf mainly response to light environment (mainly UV radiation), (R^2=0.76), while flavonoids content in the bark may have mainly acclimated to seasonal temperature changes (R^2=0.75).

In conclusion, the ecological role exited in the temporal and spatial variation of flavonoids concentration, which protected larch from damage. In complicated field conditions, accumulation of flavonoids may be related to other important factors.

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